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Isolation of Uroporphyrin from the Feces in Idiopathic Porphyria.*

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Since the first description of uroporphyrin by H. Fischer[1] this substance has been believed to be excreted only in the urine. Numerous publications concerning the excretion of porphyrins under normal or pathological conditions have failed to mention the occurrence of uroporphyrin in the feces. We have found it in the form of the zinc complex in the feces of four out of five cases of idiopathic porphyria.† Its presence was questionable in the fifth. Although uroporphyrin was readily identified in the feces of cases 1 and 3, the method of purification available at the time these were studied was inadequate to permit crystallization. By means of an improved method entailing chromatographic analysis, crystalline uroporphyrin has been isolated from cases 4 and 5. The method employed was as follows: The untreated feces was ground in a mortar with methyl alcohol saturated in the cold with hydrochloric acid gas. The methyl alcohol extract was filtered from the fecal residue on a Buchner funnel, and the residue was then extracted twice more by grinding in a mortar with an additional amount of methyl alcohol HCl. The combined methyl alcohol extract was allowed to stand overnight. It was then mixed with chloroform and several volumes of water and shaken in a separatory funnel. The chloroform fraction was washed twice with water and once with 10% NH_4OH. Shaking with an equal volume of 7% NaCl quickly breaks any emulsion. The chloroform was washed twice more with NaCl solution. It was next filtered through chloroform moistened paper and mixed with 10 volumes of petroleum ether. The filtrate was then passed through a column of Brockmann's Al_2O_3 (Merck) 1.5 x 12 cm in dimensions. The precipitate was redissolved in the least possible concentration of chloroform in petroleum ether. This was also filtered through the

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1 Fischer, H., *Z. f. physiol. Chem.*, 1915, **95**, 34.

† We are indebted to Doctors A. R. Hall and John Noble, St. Paul, Minnesota, Doctors C. E. Lynn and A. G. Plankers, Dubuque, Iowa, and Dr. W. H. Ford, Minneapolis, Minnesota, for their permission to study cases 1, 2 and 3 respectively.

Al_2O_3 column after the original 1 :10 mixture had almost completely passed through. A chromatogram as seen in Fig. 1 was then developed by elution with increasing concentrations of $CHCl_3$ in petroleum ether.

The proportions of $CHCl_3$ to petroleum ether as given for the various zones in Fig. 1, refer to the mixture used in eluting each zone. For example, the lower orange zone was completely removed with the 1 :10 mixture while the uroporphyrin zone[2] was moved but slightly, and eventually required a 1 :1 mixture for complete elution. Thus it is seen that the chromatogram in Fig. 1 is really composite since zones 1, 2, and 3 were removed before the remainder of the chromatogram was well developed.

It should be noted that a number of samples of feces from cases 4 and 5 were individually subjected to the above procedure. In some instances the total amount of pigment was relatively too large for the first Al_2O_3 column with the result that there was some over-

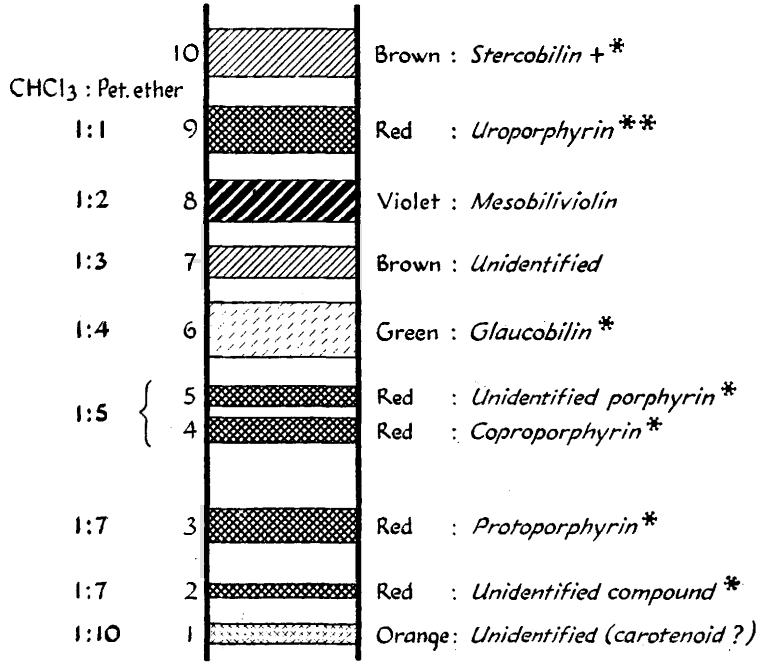

FIG. 1.

Chromatogram as developed by elution with increasing proportions of $CHCl_3$ in petroleum ether.

*Obtained in crystalline form from the feces of case 4. Present also in feces of case 5, but not crystallized.

**Crystallized from feces of both cases 4 and 5 repeatedly.

[2] Watson, C. J., *J. Biol. Chem.*, 1936, **114**, 47.

lapping of zones and the primary chromatogram in these instances was not as well defined as shown in Fig. 1. This necessitated preparation of secondary chromatograms from 2 or more overlapping zones.

The eluent from each of the zones noted in Fig. 1 was concentrated on the water bath to a volume of 1-2 cc, and hot methyl alcohol was then added. After further concentration to remove any remaining petroleum ether or chloroform, crystallization occurred, on cooling, in the fractions as indicated by the asterisks in Fig. 1. The stercobilin from zone 10 was not crystallized as the ester, but rather as the hydrochloride after saponification by heating for one-half hour in dilute NaOH, followed by acidification with HCl, and further treatment in the usual manner.[2] The glaucobilin methyl ester from case 4 crystallized directly and after 2 recrystallizations melted at 222-223°C (uncorrected). The protoporphyrin methyl ester from case 4 melted at 216-217°C (uncorrected) after 3 crystallizations from $CHCl_3$-methyl alcohol. On one occasion coproporphyrin III methyl ester was obtained from case 4, the crystals melting at 160-165°C. It may be emphasized that the esters of uro-, copro-, and protoporphyrin have always been noted on the Al_2O_3 column in the order shown in Fig. 1. Uroporphyrins were isolated repeatedly from both cases 4 and 5. These were spectroscopically identical with uroporphyrin as isolated from the urines of all 5 cases. The melting points of the methyl esters clearly indicated a mixture of the types I and III isomers. H. Fischer and Hofmann[3] have shown that uro- I ester melts at 302°C (uncorrected) while uro- III ester was found by Waldenström[4] to melt at 258°C (uncorrected). In the present study crystals from case 4 melted at 267°C (uncorrected), and from case 5 at 280°C (uncorrected). On decarboxylation of the uroporphyrins by the usual method[5] a mixture of coproporphyrins I and III was obtained in both cases. Those of case 4 were separated completely by the Al_2O_3-acetone method as previously described.[6] The copro- I ester melted at 248°C (uncorrected), and the copro- III ester at 145°C (uncorrected), and after crystallization, at 175-180°C (uncorrected). The amount of the latter was insufficient for recrystallization which may explain the somewhat elevated second melting point. The characteristic dimorphic melting of copro- III methyl ester was clearly evident, however. It may be

[3] Fischer, H., and Hofmann, H. J., *Z. f. physiol. Chem.*, 1937, **246**, 15.

[4] Waldenström, J., *Z. f. physiol. Chem.*, 1935, **233**, 1.

[5] Fischer, H., *Z. f. physiol. Chem.*, 1916, **97**, 109.

[6] Watson, C. J., and Schwartz, S., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 7.

noted further that crystals were of the typical rosette type, while those of the copro- I ester were the characteristic curved needles which were relatively insoluble in methyl alcohol.

Waldenström[4] found that uroporphyrin III is extracted by ethyl acetate from an aqueous solution at pH 3.0-3.2 (barely gray-blue to Congo paper by the addition of a very small amount of dilute HCl). The uroporphyrin III from each of the above cases behaved in this manner. It must be emphasized, however, that uroporphyrin is not unique among the porphyrins in being ether insoluble and ethyl acetate soluble, since we have encountered at least one and probably two porphyrins behaving in this manner, but nevertheless clearly distinct from uroporphyrin. These have not yet been identified and are being studied further at the present time.

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A Simple Test for Urinary Porphobilinogen.*

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The urines of patients suffering from idiopathic porphyria have often been noted to exhibit strong Ehrlich reactions, in many instances at least, not due to urobilinogen. Waldenström's studies[1, 2] clearly demonstrated that the chromogen responsible for the Ehrlich reaction in these urines is quite distinct from urobilinogen, which is of course, most often implicated in other pathological states. During the past 3 years we have had opportunity to investigate urine samples from 5 cases of so-called "acute" idiopathic porphyria.† In each instance the urine contained the zinc complex of uroporphyrin in considerable amount. The subject of zinc uroporphyria as a disease entity will be considered in a separate communication. These urines also exhibited Ehrlich reactions in varying degree, at times very

* Aided by a grant from the John and Mary R. Markle Foundation, New York City.

1 Waldenström, J., Studien uber Porphyrie, *Act. Med. Scand. Suppl.*, 1937.

2 Waldenström, J., and Vahlquist, B., *Z. f. physiol. Chem.*, 1939, **260**, 189.

† We are indebted to Doctors A. R. Hall and John Noble, St. Paul, Minnesota, Doctors C. E. Lynn and A. G. Plankers, Dubuque, Iowa, and Dr. W. H. Ford, Minneapolis, Minnesota, for their permission to study cases 1, 2 and 3 respectively.